

CLAIMS

We claim:

1. A method for constructing recombinant herpes simplex virus capable of expressing a target protein in cancer cells, comprising the steps of:
 - 5 inserting into a herpes simplex virus genome, a BAC plasmid, which has a loxP site and an FRT site and into which has been inserted at least one type of marker gene expression cassette having a structure in which a marker gene is functionally linked downstream of a promoter, between the loxP site and the FRP site;
constructing a shuttle vector into which has been respectively inserted at least
10 one type of expression cassette of a gene encoding the target protein having a structure in which the gene encoding the target protein is functionally linked downstream of a promoter, at least one type of marker gene, a loxP site and an FRP site, and inserting said shuttle vector into the loxP site of the herpes simplex virus genome using Cre recombinase so as to realize a constitution which allows
15 expression of the gene encoding the target protein and the marker gene; and,
co-infecting a host with the herpes simplex virus genome obtained in the second step and a vector capable of expressing Flp recombinase, and excising the region between the FRT sites in said genome to produce a target recombinant herpes simplex virus.
- 20 2. The method according to claim 1, wherein the second step is carried out in a liquid phase.
3. The method according to claim 1 or claim 2, wherein a γ 34.5 gene and ICP6 gene of the herpes simplex virus are deleted or inactivated prior to the first step.
4. The method according to claim 3, wherein ICP47 gene of the herpes simplex
25 virus is additionally deleted or inactivated.
5. The method according to any of claims 1 to 4, wherein the marker gene

inserted into the BAC plasmid is a gene encoding green fluorescent protein (GFP) and/or an antibiotic resistance gene.

6. The method according to any of claims 1 to 5, wherein the promoter contained in at least one type of expression cassette of a gene encoding the target protein is a promoter comprising a nucleotide sequence not present in the naturally-occurring herpes simplex virus genome.

7. The method according to any of claims 1 to 6, wherein the promoter comprising a nucleotide sequence not present in the herpes simplex virus genome is CMV promoter.

8. The method according to any of claims 1 to 7, wherein the marker gene inserted into the shuttle vector is lacZ gene and/or an antibiotic resistance gene.

9. The method according to claim 8, wherein the marker gene inserted into the shuttle vector is an antibiotic resistance gene different from the antibiotic resistance gene inserted into the BAC plasmid.

10. The method according to any of claims 1 to 9, wherein the gene encoding the target protein is one or more genes selected from the group consisting of an immunostimulatory gene, a anti-angiogenesis gene, a gene encoding a cell membrane fusion protein, and a tumor suppressor gene.

11. The method according to claim 10, wherein the immunostimulatory gene is a gene encoding one or more proteins selected from the group consisting of co-stimulatory factor, IL-12, IL-18, IL-23, IL-27 and transporter associated with antigen processing (TAP).

12. The method according to claim 10, wherein the anti-angiogenesis gene is a gene encoding one or more proteins selected from the group consisting of endostatin, angiostatin, dominant negative FGF receptor and platelet factor 4.

13. The method according to claim 10, wherein the gene encoding a cell

membrane fusion protein is a virus surface protein.

14. The method according to claim 10, wherein the tumor suppressor gene is p53 gene.

5 15. The method according to any of claims 1 to 14, wherein the shuttle vector contains a stuffer sequence.

16. The method according to claim 15, wherein the stuffer sequence is about 5000 nucleotides or more in length.

17. A recombinant herpes simplex virus constructed according to the method in any one of claims 1 to 16.

10 18. A pharmaceutical composition containing a recombinant herpes simplex virus according to claim 17.

19. The pharmaceutical composition according to claim 18, which is a therapeutic or preventive of various cancer diseases.

15 20. A method for preventing or treating cancer, comprising administration of the pharmaceutical composition according to claim 18.